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# PROTEIN ANALYSIS AND HEAVY METALS ACCUMULATION IN MUSCLES OF WILD AND FARMED NILE TILAPIA (OREOCHROMIS NILOTICUS)

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## ABSTRACT

**The present study aimed to assess the levels of some heavy metals (Cu, Fe, Pb, Zn and Cd) in muscles of wild and farmed *Oreochromis niloticus* as well as to evaluate the human hazard index associated with fish consumption. In addition, total protein, molecular weights and band counts of sarcoplasmic proteins were investigated with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method. The obtained results revealed that the accumulation of Cu has the highest value in farmed *Oreochromis niloticus* compared to wild *Oreochromis niloticus* while the highest Fe, Pb, Zn and Cd concentrations were recorded in wild *Oreochromis niloticus* compared to farmed *Oreochromis niloticus*. The calculated hazard index (HI) indicated that all metals had low HI except Pb and Cd levels in both wild and farmed fish were higher than their permissible limits for fish as a human food. There was no significant (P> 0.05) difference between wild and farmed *Oreochromis niloticus* in total protein. Wild fish predominant farmed fish in the number of separated proteins. Wild fish muscle protein showed 12 protein bands, while farmed fish muscle proteins showed 11 protein bands. Also, these bands were differed in quantitative parameters. Wild fish had unique bands (MW. 198.13, 97.92, 56.77 and 29.75) while farmed fish had unique bands (MW. 121.62, 79.05 and 26.16). The current data found that there are differences in electrophoretic pattern and heavy metals accumulation between wild and farmed *Oreochromis niloticus*.**

**Keywords:** *Protein, SDS –PAGE, Heavy metals, wild and farmed Oreochromis niloticus*

## INTRODUCTION

**Tilapia species represent the most important group of family Cichlidae that occupying the River Nile (Sharaf Eldeen and Abdel-Hamide, 2002). Heavy metals in aquatic environment are right now a noteworthy concern worldwide and positioned as major contaminating chemicals in both developed and developing countries. The particular problem related with heavy metals in the environment is their accumulation through food chain and perseverance in nature (Dimari *et al.*, 2008). Residual of heavy metal in fish muscles and its risk impacts on the health of individual are a matter of great concern to food hygienists (Desta *et al.*,**

**2012). Heavy metals are brought into the environment by a wide range of sources including; natural, anthropogenic, urbanization, industrialization and agriculture practices have additionally irritated the circumstance (Gupta *et al.*, 2009). As heavy metals are hard to be degraded, they are deposited, absorbed or incorporated in water, sediment and aquatic animals (Linnik and Zubenko, 2000; Saeed and Shaker, 2008; Ibrahim and Omar, 2013). As an outcome, fish are regularly utilized as markers of heavy metals contamination in the aquatic ecosystem because they occupy high trophic levels and are vital sustenance source they can be bioaccumulated and biomagnified via the food chain and finally assimilated by human consumers resulting in health risks (Agah *et al.*, 2009). Trace metals, for example, Zn, Cu and Fe assume a biochemical role in the life processes of all aquatic creatures; therefore, they are essential in the aquatic environment in trace amounts. In the Egyptian water system, the primary source of Cu and Pb are industrial wastes and in addition algaecides (for Cu), while that of Cd is the phosphatic composts utilized in crop farms (Mason, 2002).**

**The wild fish has to depend totally on natural food production for its nutrition while farmed fish is offered with nutrient rich foods couple with the natural productivity in the pond. These differences have direct effects on body composition, health status as well as growth of fish (Ashraf *et al.*, 2011). Protein content of fish muscles differs significantly and the variation could be due to age, feed ingestion, sex and sexual changes associated with spawning, the environment and season (Silva and Chamul, 2000). Proximate composition has been accounted for to be a good pointer of physiology required for routine examination of fisheries (Cui and Wootton, 1988). The electrophoresis of proteins is an effective procedure for creating systematic data from macromolecules. SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a strategy broadly utilized in biochemistry, criminology, genetics and molecular biology to separate proteins according to their electrophoretic mobility (Jesslin *et al.*, 2013).**

**Electrophoresis of sarcoplasmic proteins, serum proteins, liver proteins and a number of enzymes regularly has been utilized by some researchers as a guide in the species identification of fish (Hasnain *et al.*, 2005, Yilmaz *et al.*, 2007, Yilmaz *et al.*, 2008, Popoola *et al.*, 2014). Soluble proteins of muscle sarcoplasm are among the most effortless to extract and highly a rich reservoir of species specific and biochemical genetic markers. The highly water-soluble sarcoplasmic proteins comprising of glycolytic enzymes, myoglobin and other proteins present in intracellular fluid of muscle were often used for specific identification (Jesslin *et al.*, 2013). It is important to compare the genetic composition of hatchery strains with their wild populations and furthermore within strains from hatcheries as well as within wild strains (Allendorf and Ryman, 1987: Pérez *et al.*, 2001). This study aimed to assess the genetic variation of sarcoplasmic proteins by SDS-PAGE method and heavy metals of wild and farmed Nile tilapia (*Oreochromis niloticus*) as well as the health risks of the consumers after consumption of studied fish species.**

## MATERIALS AND METHODS

### Sample collection:

**Fish of wild and farmed Nile tilapia (*Oreochromis niloticus*) were obtained from the**

**local fish market (Sohag Governorate). The collected samples were brought to the laboratory in an ice box in cold condition and then washed with distilled water, and dissected for muscles and packed in polyethylene bags and stored at -20oC until analysis.**

## Heavy metal residues in muscles:

**Tissue metal analysis was performed according to Soliman (2015) with some modifications. Briefly, tissues were placed onto acid washed pre-weighed microscope slides and put into an oven at 100°C for 48 h, dried to constant weight and the tissue removed into plastic falcon tube. Samples (70-400 mg dried tissue) were then digested in 5 ml of concentrated nitric acid (69 % analytical grade) for 2 h at 70°C in a water bath (until the sample becomes clear), cooled, and then diluted to 40 ml using bidistilled water. Each sample was then analyzed for Cu, Fe, Pb, Cd and Zn by flame atomic absorption spectrophotometer (model 2380, Perkin Elmer). Analytical grade standards were used throughout, and the acidity and matrix of the standards was matched to the samples.**

## Human Risk Assessment (The hazard index):

**Human risk assessment was estimated by the method of Abdel-Khalek *et al.* (2016): The hazard index is determined according to the following equation:**

**Hazard Index = ADD/Oral RfD**

**where, ADD = average daily intake of a specific chemical over a lifetime and Oral RfD = Oral Reference dose of chemical (mg/kg/day) based on the upper level of intake mentioned earlier for each metal for an adult human with average body weight of 70 kg. The oral reference doses (RfD) for Cu, Zn, Pb, Fe, and Cd suggested by FAO/WHO (2006) was 0.14, 0.214, 0.00357, 0.643, and 0.001 mg/kg/day, respectively. HI<0.1 indicates that adverse health effects are not likely to occur. Meanwhile, if the HI was greater than or equal to 1.0, it is probably that adverse health effects will be observed.**

## Total protein content of the fish muscles:

**Sample of 100mg of muscles was homogenized in a teflon homogenizer for 3 minutes in 5ml saline then centrifuged at 3000 r.p.m for 10 minutes. The supernatant was used for determination of total protein content by Biuret reaction using Bio-diagnostic reagent Kit No.TP2020 (Gornall *et al.*, 1949).**

## Electrophoretic Protein Patterns:

## a-Sample preparation

**The fish muscles of (100mg fresh weight) from each sample were suspended in 1.0 ml lysing buffer, heated at 100˚C for 5 min., centrifuged at 10,000 r.p.m for 30 min. and 50 µl of each extracted protein treatment was mixed with sample buffer.**

## b-Preparation of Gel slab

**Gel preparation was prepared according to the method of Laemmli (1970). Resolving gel solution 12.5 % (1.5 M Tris- HCl, PH 8.8 -2 ml, 30 % acrylamide-3.2 ml, 10 % SDS-0.5 ml double distilled water-1.8 ml, TEMED-0.015 ml, ammonium per sulfate-0.5 ml) was prepared and poured in between the clamped glass plates. The plates were left undisturbed for 15 min for polymerization of the gel. After gel polymerization, overlaid water was removed and rinsed with stacking gel buffer. Now the 5% stacking gel solution (0.5M Tris-HCl, PH 6.8-2ml,**

**30% acrylamide-0.8ml, 10% SDS-0.5ml, double distilled water -1.2 ml TEMED -0.015 ml 1.5% ammonium per sulfate 0.5ml) was prepared and poured over the polymerized resolving gel, comb was inserted carefully.**

**The gel slab was left undisturbed for 15 minutes, after polymerization comb was removed carefully and the prepared samples were loaded into the wells and gel was run at 200V. The molecular weight, band counts and percentage of the individual subunits of the protein was calculated by using Gel Pro Analyzer.**

## Statistical analyses

**The results were expressed as means ± S.E. The obtained data were statistically analysed using the T-test.**

# RESULTS

## Heavy metal residues in muscles:

**The accumulation of Cu has the highest value in farmed Oreochromis niloticus compared to wild Oreochromis niloticus, contrary, the highest Fe, Pb, Zn and Cd concentrations were recorded in wild *Oreochromis niloticus* compared to farmed *Oreochromis niloticus* (Table 1 and Fig. 1).**

**Table1. Heavy metals level (µg/g dry weight) in muscle of wild and farmed Oreochromis niloticus.**

| | Cu | Fe | pb | Zn | Cd |
|---|---|---|---|---|---|
| Wild | 0.1±0.09 | 60.34±24.16* | 92.50±9.01* | 5.04±0.63* | 5.04±0.63* |
| Farmed | 0.81±0.17* | 35.27±20.59 | 35.98±8.85 | 2.57±0.61 | 2.57±0.61 |

*** Statistically significant difference (T test, P < 0.05). n=4**

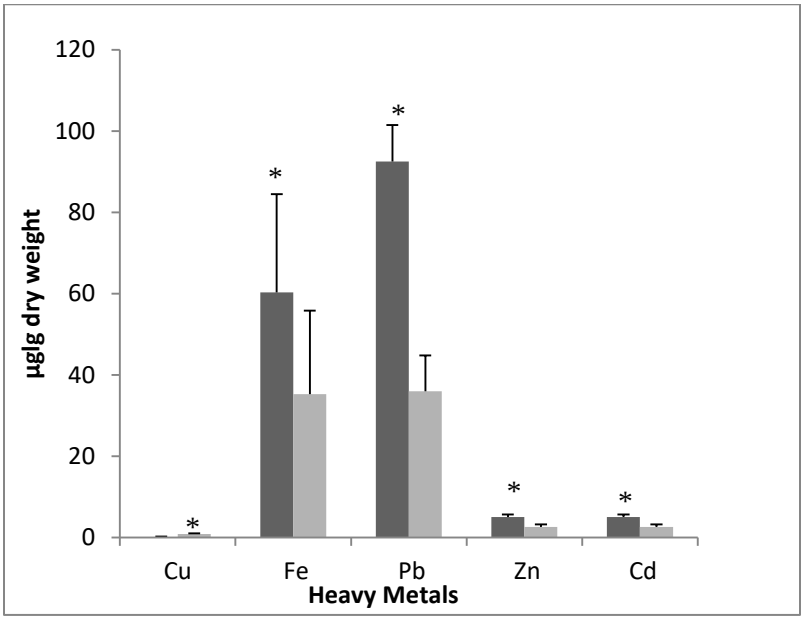


**Fig.1. Heavy metals (µg/g dry weight) in muscle of wild and farmed Oreochromis niloticus.**

## Hazard index (HI):

**Based on the values of HI at mean ingestion rate for normal adult , there was no adverse health effect is likely to occur (all values of HI<1 ) except Pb and Cd levels in both wild and farmed *Oreochromis niloticus* were higher than their permissible limits for fish as a human food (Table 2).**

**Table 2. Hazard index for muscle of wild and farmed Oreochromis niloticus.**

| | Cu | Fe | pb | Zn | Cd |
|---|---|---|---|---|---|
| Wild | 0.10 | 0.02 | 4.07 | 0.07 | 14.53 |
| Farmed | 0.05 | 0.01 | 1.93 | 0.03 | 6.88 |

## Total protein content of the fish muscles:

**The total protein content in muscle of wild and farmed *Oreochromis niloticus* recorded average value 6.3 ± 1.5 and 6.7 ± 0.23, respectively. There was no significant (P > 0.05) difference between wild and farmed fish (Table 3).**

**Table 3. The total protein content (mg/g wet weight) in muscle of wild and farmed Oreochromis niloticus.**

| | Protein (mg/g wet weight) |
|---|---|
| Wild | 6.3 ± 1.5 |
| Farmed | 6.7 ± 0.23 |

*** Statistically significant difference (T test, P < 0.05). n=6**

## Electrophoretic Protein Patterns

**Eelectrophoretic pattern of muscle proteins in wild and farmed *Oreochromis niloticus* were shown in (Fig. 2). Wild *Oreochromis niloticus* predominant farmed *Oreochromis niloticus* in the number of separated proteins. Wild *Oreochromis niloticus* muscle proteins showed 12 protein bands, whereas farmed *Oreochromis niloticus* muscle proteins showed 11 protein bands (Fig. 2 and Table 4). Wild *Oreochromis niloticus* and farmed *Oreochromis niloticus* muscle proteins showed several bands; these bands were differed in quantitative parameters (Table 4). Wild *Oreochromis niloticus* had unique bands (MW, 198.13, 97.92, 56.77 and 29.75) while farmed *Oreochromis niloticus* had unique bands (MW, 121.62, 79.05 and 26.16) (Table 4).**

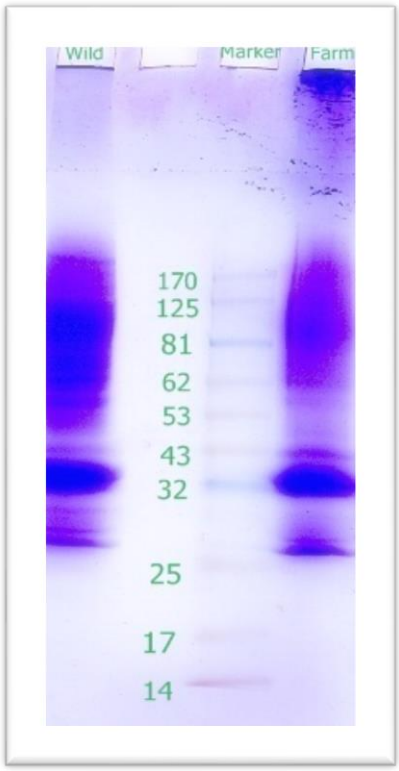


**Fig. 2. Comparison of sarcoplasmic proteins of wild and farmed Oreochromis niloticus.**

**Table 4. Molecular weights of sarcoplasmic protein bands of wild and farmed Oreochromis niloticus.**

| Marker (kD) | Wild (kD) | Farmed (kD) |
|---|---|---|
| - | **198.13** | - |
| 170 | **179.38** | **171.88** |
| 125 | - | **121.62** |
| - | **97.92** | - |
| - | **91.15** | **91.15** |
| 81 | - | **79.05** |
| 62 | **63.46** | **63.46** |
| - | **56.77** | - |
| 53 | **53.29** | **55.90** |
| 43 | **43.28** | **41.66** |
| 32 | **33.66** | **32.33** |
| - | **29.75** | -- |
| - | **27.96** | **27.78** |
| - | **26.88** | **27.15** |
| - | - | **26.16** |
| 25 | - | - |
| 17 | - | - |
| 14 | - | - |

## DISCUSSION

**Among the myriad pollutants discharged into aquatic ecosystems, heavy metals have received considerable attention due to their toxicity, long-term persistence, bioaccumulation, and biomagnifications at various trophic levels (Ololade *et al*., 2008). Fish may ingest dissolved elements and trace metals and afterward accumulate them in different tissues in significant amounts above those found in their environment, thus displaying elicited toxicological impacts (McCarthy and Shugart, 1990). Bioaccumulation of metals in tissues differs from metal to metal and varies in different organisms additionally among various organs of the same organism (Watanabe *et al.*, 2003; Masoud *et al.*, 2007). Since most trace metals tend to accumulate in the various body organs, these metals are dangerous for fish and thus they lead to serious problems in both humans and animals (Marzouk, 1994). In the present study, the accumulation of Cu has the highest value in farmed *Oreochromis niloticus* compared to wild *Oreochromis niloticus*. Cu compounds are both effective algaecide and parasiticide and are prophylactically used to control fish diseases and parasites in freshwater ponds and aquaculture operations (Moore *et al.*, 1984). While the highest Fe, Pb, Zn and Cd concentrations were recorded in wild *Oreochromis niloticus* compared to farmed *Oreochromis niloticus*. The high Cd, Fe and Zn concentrations could be attributed to the industrial effluents discharged directly in the River Nile and its branches, as previously reported by El-Naggar *et al.* (1998), Haggag *et al.* (1999) and Salah El-Deen (1999). Also, the higher concentration of Pb may be attributed to the fuel effluents from the cruise boats, this explanation is in agreement with Shenouda *et al.* (1992) and Begum *et al.* (2005) who reported that water crafts with gasoline motors lead to more polluted water, plankton, algae and plants with Pb metal.**

**Hazard index is utilized to perceive and evaluate the potentially harmful metals in the edible tissues of fish that confronting distinctive pollution levels. Bioaccumulation of metals in the edible tissues is a useful tool for examining the biological responsibility of those metals that present at elevated levels other than assessment of public health risk (Bastami *et al.*, 2015). In present study, the values of HI<1 for all metals except Pb and Cd levels in both wild and farmed *Oreochromis niloticus* were higher than their permissible limits for fish as a human food. However, This is in agreement with Abdel-Khalek *et al.* (2016) who stated that the values of HI<1 for all metals except some HI values (ex. Cd in case of habitual fish eaters at Sabal site) were higher than other values along the studied sites showing alarming values. The variations in the chemical composition of fish are firmly related to the environment of rearing in ponds or nature and completely depend on feed ingestion (Sulieman and James, 2011). Farmed fish have an advantage over wild caught fishery products since they are produced and gathered under controlled conditions, and hence the dangers related with fish utilization may be decrease (FAO, 1998).**

**The total protein content in muscle of wild and farmed *Oreochromis niloticus* recorded average value 6.3 ± 1.5 and 6.7 ± 0.23, respectively. There was no significant (P>0.05) difference between wild and farmed *Oreochromis niloticus*. The protein contents of farm raised grass carp and silver carp were significantly (P<0.05) higher than**

**those caught from the wild (Ashraf *et al.*, 2011). On other hand, Obaroh *et al.* (2015) found that the highest crude protein as observed in the wild juvenile fish, while the lowest was recorded for cultured adult fish. Wild Oreochromis niloticus predominant farmed Oreochromis niloticus in the number of separated proteins. Wild and farmed fish muscle proteins were separated into 12 and 11 protein bands, respectively and these bands were differed in quantitative parameters. Wild Oreochromis niloticus had unique bands (MW. 198.13, 97.92, 56.77 and 29.75) while farmed Oreochromis niloticus had unique bands (MW. 121.62, 79.05 and 26.16). The overlapped MW of some of the bands indicates that some proteins are common to the individual fish sample. This could be a result of belonging to the same species or taxa. The presence of unique band gives an indication that certain soluble protein is common to these samples. This unique band could be used as a marker in delineating wild population (Popoola et al., 2014). Suzuki and Van Ngan (1990) found ontogenetic variations in the relative concentration of bands of eye-lens and skeletic muscle proteins in the six species of catfishes. Also, Knuutinen and Harjula (1998) found differences in band numbers and MWs among 16 freshwater fish species. Moreover, Zowail and Baker (1998) observed differences among the serum proteins of five species of freshwater fish (Sarotheredon galilaeus, Tilapia zillii, Orecohromis niloticus, Clarias lazera, and Barbus bynni). According to genetic similarity and distance between characid and cyprinid fishes, they closely related to each other (Shahin, 1999). Furthermore, Hasnain et al. (2005) found varations among soluble muscle proteins in 4 fish species (Channa gachua and Channa striatus, C. marulus C. punctatus). These data showed that the total number of skeletal muscle protein bands could differ among classes, species, or subspecies. In addition, Yilmaz *et al.* (2007) found differences in the serum proteins between *Leuciscus cephalus, Acanthobrama marmid* and *Chondrostoma regium* (Cyprinidae). Also, Yilmaz *et al*. (2008) observed varations in sarcoplasmic proteins between *Orthrias tigris, Orthrias angorae bureschi, Orthrias panthera* and CobitisTaenia. Finally, Popoola *et al.* (2014) found a genetic variations between wild and cultured *C. gariepinus*.**

**In conclusion, the present study found that there are differences in electrophoretic pattern and heay metals accumulation between wild and farmed *Oreochromis niloticus* with Pb and Cd levels in both wild and farmed *Oreochromis niloticus* were higher than their permissible limits for fish as a human food. Accordingly, the author's advice to decrease the leaching as well as running off of heavy metals (especially, Pb and Cd) into River Nile or fish farm.**

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# تحليل البروتينات و تراكم العناصر الثقيلة فى عضلات البلطى النيلى البرى و المستزرع

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## الملخص العربي

**هذه الدراسة هدفت الى تقييم مستويات بعض العناصر الثقيلة (النحاس، الحديد، الرصاص، الزنك و الكادميوم) في عضلات اسماك البلطي النيلي البرى و المستزرع وكذلك لتقييم مؤشر الخطر البشري المرتبطة باستهلاك هذه الأسماك. بالإضافة إلى قياس محتوى البروتين الكلي، الأوزان الجزيئية و عدد الحزم بواسطة التفريد الكهربائي لبروتين العضلات باستخدام طريقة التفريد الكهربائي النشوى الهلامى (SDS–PAGE).**

**ووجدت نتائج الدراسة أن مستوى تراكم أعلى في البلطي النيلي المستزرع مقارنة بالبلطي النيلي البري في حين تم تسجيل أعلى تركيز للحديد والرصاص والزنك والكادميوم في أسماك البلطي النيلي البري مقارنة بالبلطي النيلي المستزرع. أشار مؤشر الخطر البشري أن جميع العناصر قيمتها منخفضة باستثناء مستويات الرصاص والكادميوم في كلا من أسماك البلطي النيلي البري والمستزرع حيث كانت أعلى من الحدود المسموح بها للأسماك كغذاء بشري. ومما هو جدير بالذكر انه لم يكن هناك فرق معنوى بين عضلات اسماك البلطي النيلي البرى و المستزرع في البروتين الكلي. و ان البلطي النيلي البرى يسود البلطي النيلي المستزرع في عدد الحزم المنفصلة. حيث اظهرت بروتينات عضلات البلطي البري (١٢) حزمة، في حين اظهرت عضلات البلطي المستزرع ١١حزمة. واختلفت هذه الحزم في المعلمات الكمية (الأوزان الجزيئية ). وكان للبلطي النيلي البرى حزم فريدة من نوعها (وزنها الجزيئى : ١٩٨.١٣، ٩٧.٩٢، ٥٦.٧٧ ، ٢٩.٧٥)، في حين كان االبلطي النيلي المستزرع حزم فريدة من نوعها (وزنها الجزيئى : ١٢١.٦٢، ٧٩.٠٥ ، ٢٦.١٦). وخلص البحث إلي وجود اختلافات في نمط التفريد الكهربائي للبروتين و تراكم العناصر الثقيلة بين عضلات اسماك البلطي النيلي البرى والمستزرع.**